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Robust *de novo* designed homotetrameric coiled coils

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Supporting Information Placeholder

ABSTRACT: *De novo* designed protein domains are increasingly being applied in biotechnology, cell biology and synthetic biology. Therefore, it is imperative that these proteins are robust to superficial changes, *i.e.*, small changes to their amino-acid sequences should not cause gross structural changes. In turn, this allows properties such as stability and solubility to be tuned without affecting structural attributes like tertiary fold and quaternary interactions. Reliably designed proteins with predictable behaviors may then be used as scaffolds to incorporate function, *e.g.*, through the introduction of features for small-molecule, metal or macromolecular binding, and enzyme-like active sites. Generally, achieving this requires the starting protein fold to be well understood. Herein, we focus on designing α -helical coiled coils, which are well studied, widespread and often direct protein-protein interactions in natural systems. Our initial investigations reveal that a previously designed parallel, homotetrameric coiled coil, CC-Tet, is not robust to sequence changes that were anticipated to maintain its structure. Instead, the alterations switch the oligomeric state from tetramer to trimer. To improve the robustness of designed homotetramers, additional sequences based on CC-Tet were produced and characterized in solution and by X-ray crystallography. Of these updated sequences, one is robust to truncation and to changes in surface electrostatics; we call this CC-Tet*. Variants of the general CC-Tet* design provide a set of homotetrameric coiled coils with unfolding temperatures in the range 40 °C to >95 °C. We anticipate that these will be of use in applications requiring robust and well defined tetramerization domains.

It is now possible to design a variety of protein structures *de novo* using computational and rational approaches.^{1,2} Whilst much of this work has been done with the aim of exploring accessible protein topologies, attention is turning to ensuring protein designs can address the challenges they will face in practical applications.³ For example, it is important that they can tolerate superficial sequence changes without losing structural specificity. This is because, once a target structure has been achieved it may be augmented to give it new properties or to incorporate new functions.

One family of protein structure that can be designed relatively reliably is the α -helical coiled coil (CC). In these

assemblies two or more α helices wrap around one another to form super-helical bundles.⁴ This is directed by 7-residue sequence repeats called heptads, in which the residues are labelled *abcdefg* (Figure 1A). The specific structure adopted by a CC is determined by the heptad sequence. In particular, the identities of hydrophobic-core residues at *a* and *d* discriminate between dimeric, trimeric and tetrameric structures.⁵⁻⁷ The *e* and *g* residues also influence oligomer state and topology.^{8,9} Beyond this, the peripheral *b*, *c* and *f* residues generally do not significantly impact overall CC structure, though they may influence stability, solubility or interactions with other molecules.^{10,11} CC stability may also be adjusted by changing the length of the constituent helices.^{12,13} More ambitious changes include the incorporation of sites for enzyme-like activity and ligand/protein binding.^{14,15} When these features are added to existing scaffolds it is important that oligomeric state or relative helix orientation are unaffected. However, some sequence changes can have unpredictable effects and structural plasticity has been noted in both natural and *de novo* CC assemblies.^{16,17}

Here we investigate the structural integrity of *de novo* designed homotetrameric CCs containing all-parallel helices. These CCs are especially useful as protein-protein interaction domains in cell and synthetic biology and biotechnology.¹⁸⁻²¹ Tetramers also have relatively large hydrophobic cores making them highly stable, so they may be able to tolerate a wider range of sequence modifications without becoming excessively destabilized or losing structural specificity. Indeed, non-CC 4-helix bundles have been used for some time as scaffolds into which functions can be introduced.²²

The key guidelines for designing tetrameric CCs are to place leucine (Leu, L) at the *a* position of the heptad and isoleucine (Ile, I) at *d*.^{6,7} This creates a hydrophobic seam though which the helices interact and forms the core of the CC. This is often flanked by complementary charged residues like glutamate (Glu, E) and lysine (Lys, K) at *e* and *g*. Conversely, expanding the hydrophobic seam by placing small hydrophobic residues such as alanine (Ala, A) at *e/g* can have profound effects on structure, potentially resulting in larger parallel oligomers,^{8,9,23} or in antiparallel tetramers where the relative helix orientation changes.^{16,24}

As part of a basis set of *de novo* CC modules,⁶ previously we applied these principles to design a parallel CC tetramer, CC-Tet,⁸ with the sequence repeat, L-A-A-I-K-X-E (*a-b-c-d-e-f-g*,

Table 1. Peptide sequences.

Peptide	Sequence
<i>g abcdefg abcdefg abcdefg abcdefg ab</i>	
CC-Tet	
CC-Tet	Ac - G E LAAIKQE LAAIKKE LAAIKWE LAAIKQ GAG - NH ₂
CC-Tet-KE	Ac - G K LAAIEQK LAAIEKK LAAIEWK LAAIEQ GAG - NH ₂
CC-Tet-3	Ac - G E LAAIKKE LAAIKWE LAAIKQ G - NH ₂
Class 1	
1-EK-4	Ac - G AIKKE LAAIKKE LAAIKWE LAAIKKE LA G - NH ₂
1-KE-4	Ac - G AIEQK LAAIEQK LAAIEWK LAAIEQK LA G - NH ₂
1-EK-3	Ac - G AIKKE LAAIKWE LAAIKKE LA G - NH ₂
Class 2	
2-EK-4	Ac - G EIKQQ LAEIKQQ LAEIKWQ LAEIKQQ LA G - NH ₂
2-KE-4	Ac - G KIEQQ LAKIEQQ LAKIEWQ LAKIEQQ LA G - NH ₂
2-EK-3	Ac - G EIKQQ LAEIKWQ LAEIKQQ LA G - NH ₂
Class 3	
3-EK-4	Ac - G AIQQE LKAIQQE LKAIQWE LKAIQQE LK G - NH ₂
3-KE-4	Ac - G AIQQK LEAIQQK LEAIQWK LEAIQQK LE G - NH ₂
3-EK-3	Ac - G AIQQE LKAIQWE LKAIQQE LK G - NH ₂
Class 4	
4-EK-4	Ac - G KIQKQ LEKIQKQ LEKIQWQ LEKIQKQ LE G - NH ₂
4-KE-4	Ac - G EIQKQ LKEIQKQ LKEIQWQ LKEIQKQ LK G - NH ₂
4-EK-3.5-N	Ac - G Q LEKIQKQ LEKIQWQ LEKIQKQ LE G - NH ₂
4-KE-3.5-N	Ac - G Q LKEIQKQ LKEIQWQ LKEIQKQ LK G - NH ₂
4-EK-3.5-C	Ac - G KIQKQ LEKIQKQ LEKIQWQ LEKIQK G - NH ₂
4-KE-3.5-C	Ac - G EIQKQ LKEIQKQ LKEIQWQ LKEIQK G - NH ₂
4-EK-3	Ac - G KIQKQ LEKIQWQ LEKIQKQ LE G - NH ₂
4-KE-3	Ac - G EIQKQ LKEIQWQ LKEIQKQ LK G - NH ₂

The class-1–4 peptides were named for their heptad arrangement (1–4), charge pattern (EK; or KE, where charged residues are inverted) and number of heptads (3, 3.5 or 4). The heptad register is indicated above the sequences. All sequences contained *N*- and *C*-terminal glycine residues (not included in heptad register assignment) and were *N*-terminally acylated and *C*-terminally amidated.

where X is tryptophan [Trp, W], K or glutamine [Gln, Q]) (Figure 1A). While the basis set CCs can be used as “off-the-shelf” parts in various applications,^{18–21,25} as discussed above it may be desirable to alter these designs to give them more bespoke properties. Therefore, to determine whether it could tolerate sequence changes predicted to maintain its general topology, we probed CC-Tet sequence variants as follows.

First, a charge-swapped variant of CC-Tet was made with the E/K residues at *g* and *e* inverted to give CC-Tet-KE (Table 1). By circular dichroism (CD) spectroscopy CC-Tet-KE was highly α helical, but less thermally stable than CC-Tet (Figure 1B,C; Table S2). However, when the oligomeric state was investigated by analytical ultracentrifugation (AUC), CC-Tet-KE formed a trimer, rather than the anticipated tetramer (Figures 1D and S25).

In addition, CC-Tet was not robust to truncation: with the aim of modulating its thermal stability, we removed the *N*-terminal heptad of CC-Tet to give a three-heptad peptide, CC-Tet-3 (Table 1). CD spectroscopy showed that this was partially α helical with a much lower thermal stability than CC-Tet

(Figure 1B,C; Table S2). However, AUC experiments for CC-Tet-3 returned trimer molecular weights (Figures 1D and S26).

Therefore, the original CC-Tet homotetramer is not robust to sequence changes that were not expected to affect oligomeric state. Though this *de novo* CC can be used as-is, it may not be optimal for applications where the CC sequence will be altered. Variants of this particular module should be used with caution.

To design more reliable homotetramers, several alternative sequences were considered (Figure 2A–D; Table 1). Compared with dimers and trimers, homotetrameric CCs have wider helix-helix interfaces with CC-defining knobs-into-holes packing that extends past *a* and *d* positions to involve side chains at *g* and/or *e*.^{26,27} Therefore, we reasoned that the *g* and *e* residues may play a role in specifying tetrameric CCs. To test this, the *e* and *g* positions were made combinations of Glu, Lys and the uncharged, polar residue Gln. We avoided small residues like Ala because of the aforementioned possibility of forming alternative structures.^{8,9,16,23} Furthermore, the new designs were in *c*-register rather than *g*-register, *i.e.*, the CC sequences began

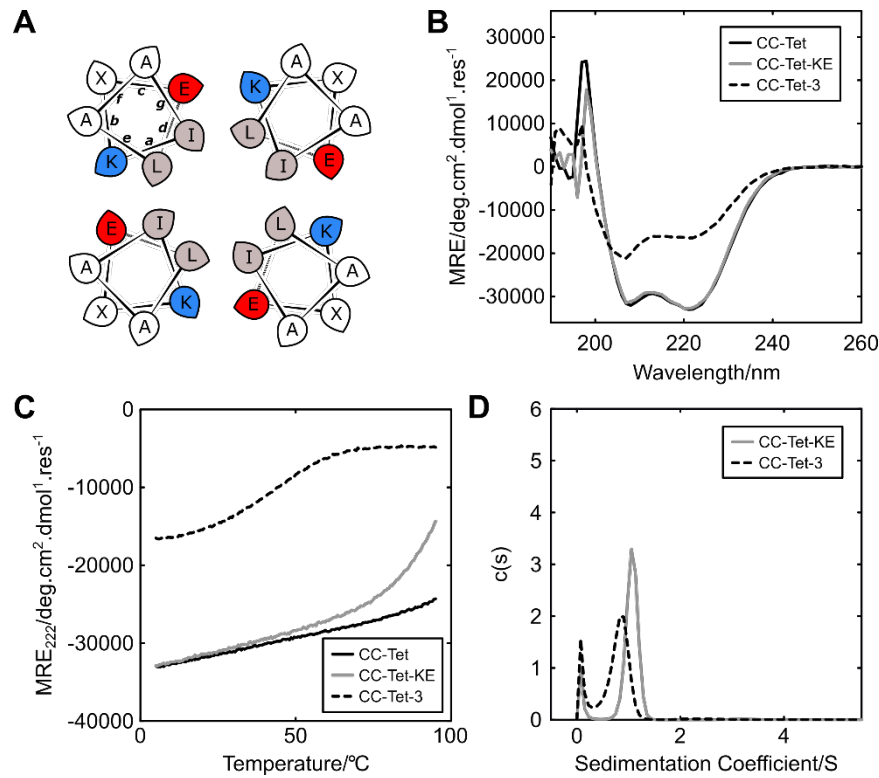


Figure 1. Biophysical characterization of CC-Tet variants. (A) Helical wheel for CC-Tet annotated with heptad positions. (B) CD spectra for CC-Tet, CC-Tet-KE and CC-Tet-3 measured at 5 °C and 10 μ M peptide concentration. (C) Variable temperature CD measurements for CC-Tet, CC-Tet-KE and CC-Tet-3 monitoring MRE₂₂₂ from 5–95 °C at 10 μ M peptide concentration. (D) SV experiments for CC-Tet-KE and CC-Tet-3, returning molecular weights of 10.5 and 7.2 kDa, respectively. All measurements were performed in phosphate-buffered saline (PBS, pH 7.4).

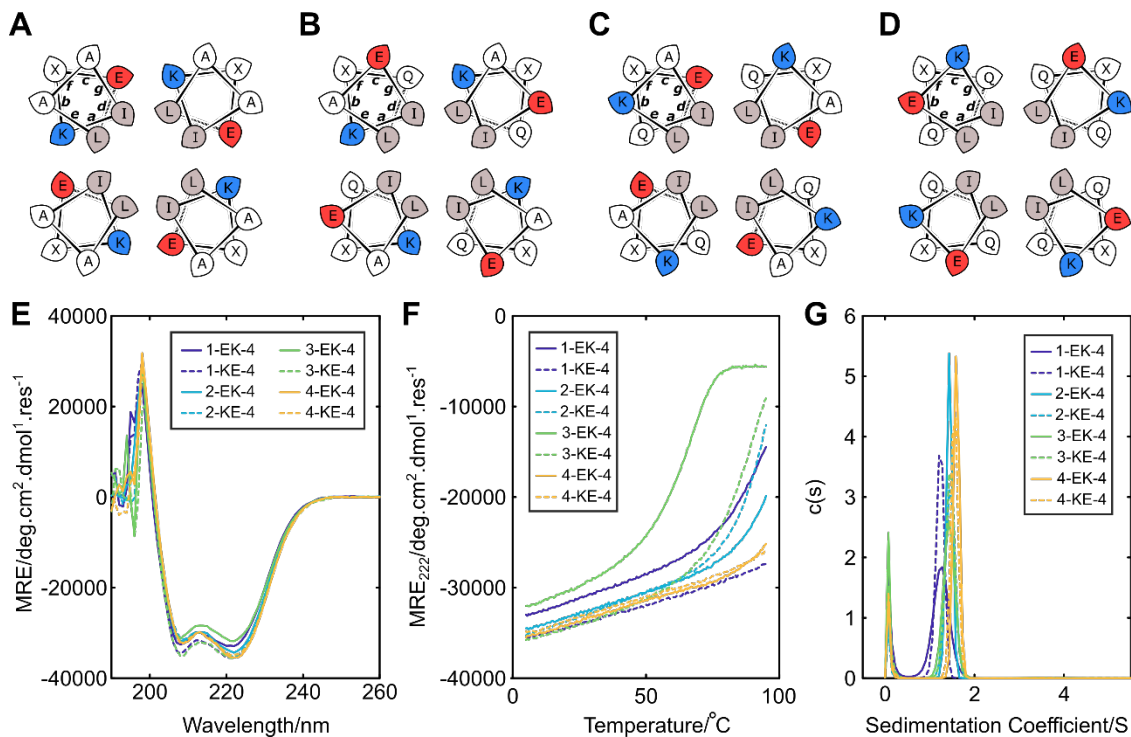


Figure 2. Design and biophysical characterization of class-1–4 peptides. Helical wheels for (A) 1-EK-4, (B) 2-EK-4, (C) 3-EK-4 and (D) 4-EK-4. (E) CD spectra for 1-EK-4, 1-KE-4, 2-EK-4, 2-KE-4, 3-EK-4, 3-KE-4, 4-EK-4 and 4-KE-4 measured at 5 °C and 10 μ M peptide concentration. (F) Variable temperature CD measurements for the above peptides monitoring MRE₂₂₂ from 5–95 °C at 10 μ M peptide concentration. (G) SV experiments for the above peptides, returning molecular weights of 12.9, 11.3, 13.4, 14.4, 14.5, 13.7, 15.3 and 15.4 kDa, respectively. All measurements were performed in PBS (pH 7.4).

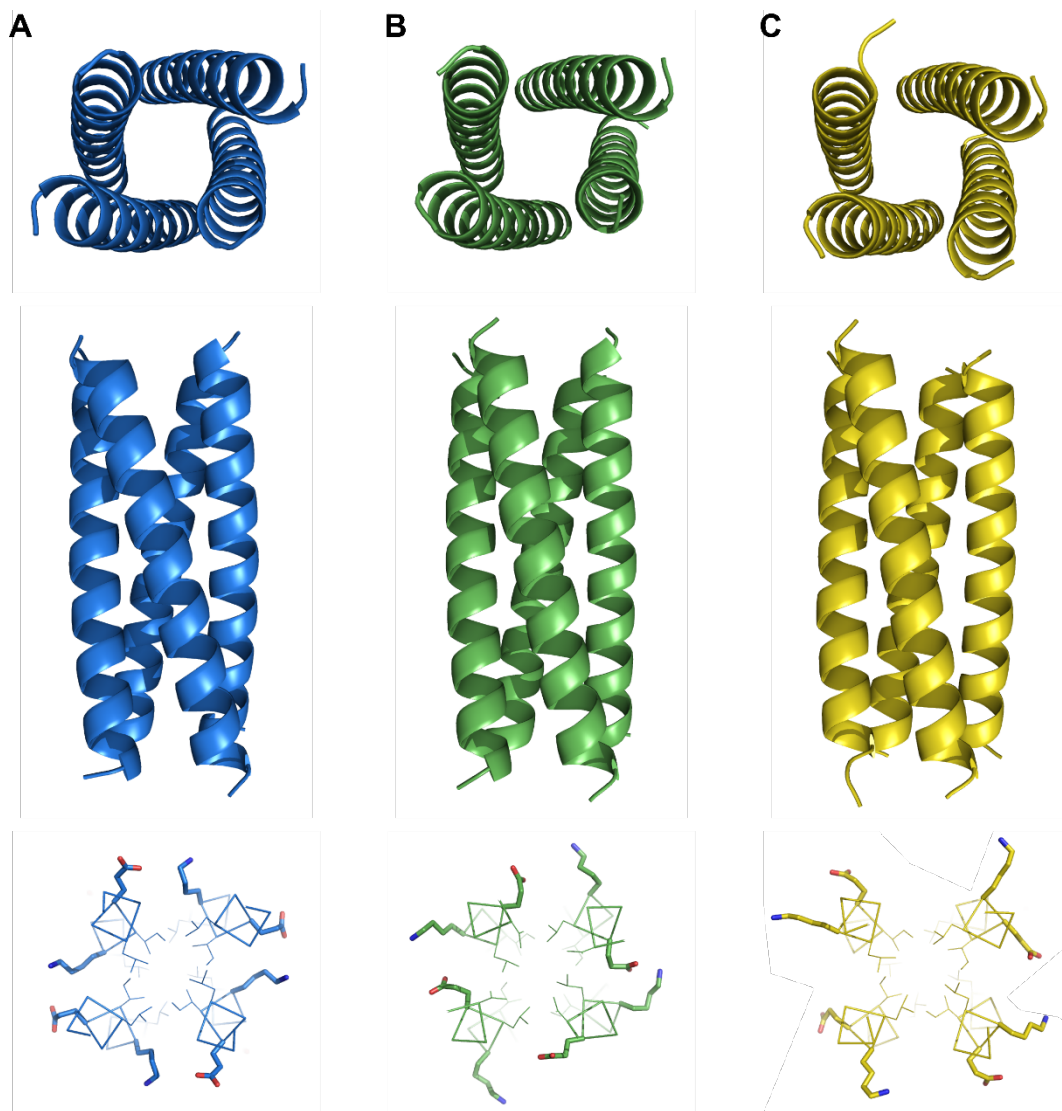


Figure 3. X-ray crystal structures of (A) 2-EK-4, (B) 3-EK-4 and (C) 4-KE-4. Structures are shown from the *N* termini (top) and from the side with the *N* termini towards the top (middle). Cut-through views (bottom) are shown from the *N* termini with main chains shown as ribbons, core side chains as lines and Glu/Lys residues as sticks. Collection and refinement statistics are in Tables S3–S6.

at a *c* position, rather than *g*. This was done to maximise interhelical Coulombic interactions, and to be consistent with our own growing set of standard higher-order *de novo* coiled-coil peptides.⁹ Therefore, all peptides herein were designed with this register to maintain this consistency. Overall, 4 classes were explored: class 1, with Glu and Lys at *e* and *g*; class 2, with Gln at *g*; class 3, with Gln at *e*; and class 4, with Gln at both *e* and *g*. In classes 2–4, the displaced Glu/Lys residues were moved to *b* and/or *c* sites to maintain possible favorable interhelical Coulombic interactions. Including the charge-swapped variants, eight four-heptad-long peptides were designed (Table 1).

Considering class 1 first, 1-EK-4 is analogous to CC-Tet apart from the change in register. 1-EK-4 formed a highly α helical, thermally stable tetramer (Figures 2E–G and S27; Table S2). However, though α helical, 1-KE-4 formed a mixture of trimers and tetramers in solution (Figure S28). Furthermore, a 3-heptad variant of 1-EK-4, 1-EK-3, was one of the most thermally destabilized structures in this study (Figure S15; Tables 1 and S2). Thus, this heptad arrangement, L-A-A-I-K/E-X-E/K (*a-b-c-d-e-f-g*, X = W, K or Q), is not compatible with reliable tetramer formation, regardless of sequence register.

For classes 2 and 3, where Gln is either at all *g* or all *e* sites, respectively, the 4-heptad variants formed thermally stable α -helical tetramers in solution (Figures 2E–G and S29–S32; Table S2). Moreover, the X-ray crystal structures of 2-EK-4 and 3-EK-4 were solved to 1.7 and 1.1 Å resolution, respectively, and both revealed parallel, blunt-ended, homotetrameric CCs (Figure 3A,B; Tables S4 and S5). However, truncating these peptides to give 2-EK-3 and 3-EK-3 compromised their oligomeric-state specificities (Figures S16 and S17; Tables 1 and S2). For example, though partly folded with a T_M of 37 °C, 2-EK-3 was largely trimeric in solution (Figure S35).

Finally, the class 4 peptides, with Gln at both *e* and *g*, showed the most robust and predictable behavior of all the designs. 4-EK-4 and 4-KE-4 were both thermally stable α -helical tetramers in solution (Figures 2E–G, S33 and S34; Table S2), and the X-ray crystal structure of 4-KE-4 revealed an all-parallel, blunt-ended, tetrameric CC (Figure 3C; Table S6). The 3-heptad versions of 4-EK-4 and 4-KE-4 also formed homotetramers (Figures S36 and S37), making L-K/E-K-I-Q-X-Q (*a-b-c-d-e-f-g*, X = W or K) the only heptad arrangement that can tolerate charge inversion and truncation without losing oligomeric-state specificity.

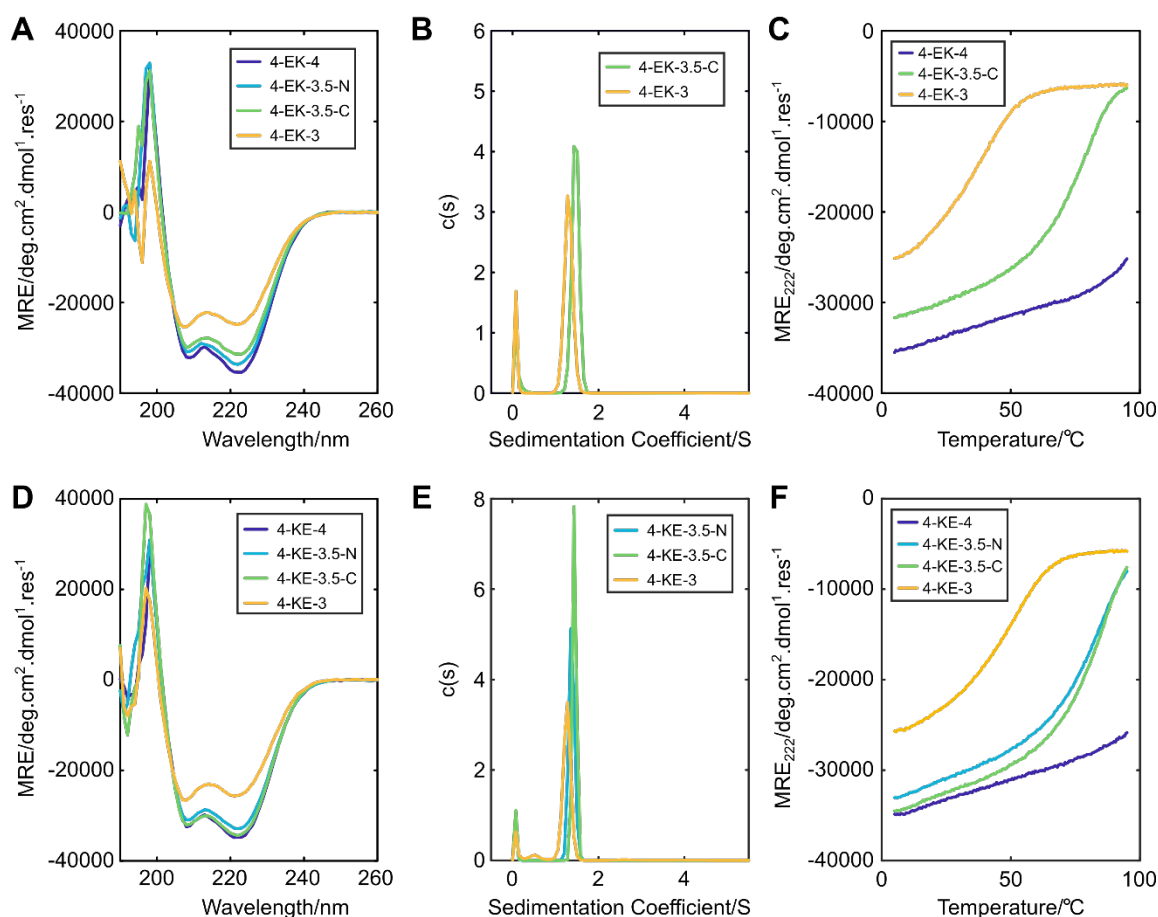


Figure 4. Biophysical characterization of class-4 peptides. (A) CD spectra for 4-EK-4, 4-EK-3.5-N, 4-EK-3.5-C and 4-EK-3 measured at 5 °C. (B) SV experiments for 4-EK-3.5-C and 4-EK-3, returning molecular weights of 14.0 and 11.3 kDa, respectively. 4-EK-3.5-N precipitated. (C) Variable temperature CD measurements for 4-EK-4, 4-EK-3.5-C and 4-EK-3, monitoring MRE₂₂₂ from 5–95 °C. (D) CD spectra for 4-KE-4, 4-KE-3.5-N, 4-KE-3.5-C and 4-KE-3 measured at 5 °C. (E) SV experiments for 4-KE-3.5-N, 4-KE-3.5-C and 4-KE-3, returning molecular weights of 13.2, 13.1 and 10.7 kDa, respectively. (F) Variable temperature CD measurements for 4-KE-4, 4-KE-3.5-N, 4-KE-3.5-C and 4-KE-3 monitoring MRE₂₂₂ from 5–95 °C. CD measurements were performed at 10 μM peptide concentration. All measurements were performed in PBS (pH 7.4).

Subsequently, 3.5-heptad versions of the class-4 peptides were designed in order to deliver a set of homotetrameric CC modules with a range of thermal stabilities (Table 1). 4-EK-3.5-N and 4-EK-3.5-C were based on 4-EK-4 with half heptads removed from the *N* or *C* terminus, respectively; 4-KE-3.5-N and 4-KE-3.5-C were similar but based on 4-KE-4. All four 3.5-heptad peptides were α helical (Figure 4A,D; Table S2). All were tetrameric in solution except 4-EK-3.5-N, which precipitated (Figure 4B,E and S38–S40). Moreover, the 3.5-heptad variants had T_M s in the range 80–90 °C. Conversely, the 4-heptad variants were hyperthermally stable and did not unfold, and the 3-heptad variants had T_M s of \approx 40 °C and \approx 52 °C (Figure 4C,F; Table S2).

In summary, the class-4 heptad repeat, L-K/E-E/K-I-Q-X-Q (*a-b-c-d-e-f-g*, X = W or K), is the most robust of the investigated designs, tolerating both truncation and changes in surface electrostatics. It is not completely clear why this should be. One possibility is that because Gln is uncharged, when placed at *e* and *g* it packs better to make knobs-into-holes interactions^{26,27} compared with Glu or Lys at these sites. In this way, Gln residues may contribute better to tetramer specification. Indeed, analysis the X-ray structures that we have obtained does provide some evidence for this.

We recommend the class-4 designs for use in applications where additional, minor modifications to the designed CC sequences are required, as they are more accommodating of variation than the original CC-Tet.⁶ We name this class of peptides CC-Tet*. We anticipate they will be useful scaffolds

that could be augmented for supramolecular assembly, or by introducing sites for binding, catalysis or post-translational modification. Furthermore, the CC-Tet* peptides of different lengths constitute a set of well-characterized homotetrameric CCs with a range of thermal stabilities. These may be of use in cell biology, synthetic biology and biotechnology applications where quaternary protein interactions with variable stabilities are required.

ASSOCIATED CONTENT

Supporting information:

Materials and methods, supplementary figures S1–S40, supplementary tables S1–S6 and supplementary references.

Accession codes:

2-EK-4 PDB 6XXZ; 3-EK-4 PDB 6XY0; 4-KE-4 PDB 6XY1.

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REFERENCES:

- (1) Regan, L., Caballero, D., Hinrichsen, M. R., Virrueta, A., Williams, D. M., and O'Hern, C. S. (2015) Protein Design: Past, Present, and Future. *Biopolymers* 104, 334-350.
- (2) Huang, P. S., Boyken, S. E., and Baker, D. (2016) The coming of age of de novo protein design. *Nature* 537, 320-327.
- (3) Gainza-Cirauqui, P., and Correia, B. E. (2018) Computational protein design - the next generation tool to expand synthetic biology applications. *Curr. Opin. Biotech.* 52, 145-152.
- (4) Lupas, A. N., and Bassler, J. (2017) Coiled Coils - A Model System for the 21st Century. *Trends Biochem. Sci.* 42, 130-140.
- (5) Woolfson, D. N. (2017) Coiled-Coil Design: Updated and Upgraded. *Subcell. Biochem.* 82, 35-61.
- (6) Fletcher, J. M., Boyle, A. L., Bruning, M., Bartlett, G. J., Vincent, T. L., Zaccai, N. R., Armstrong, C. T., Bromley, E. H. C., Booth, P. J., Brady, R. L., et al. (2012) A Basis Set of de Novo Coiled-Coil Peptide Oligomers for Rational Protein Design and Synthetic Biology. *ACS Synth. Biol.* 1, 240-250.
- (7) Harbury, P. B., Zhang, T., Kim, P. S., and Alber, T. (1993) A Switch between 2-Stranded, 3-Stranded and 4-Stranded Coiled Coils in Gcn4 Leucine-Zipper Mutants. *Science* 262, 1401-1407.
- (8) Zaccai, N. R., Chi, B., Thomson, A. R., Boyle, A. L., Bartlett, G. J., Bruning, M., Linden, N., Sessions, R. B., Booth, P. J., Brady, R. L., et al. (2011) A de novo peptide hexamer with a mutable channel. *Nat. Chem. Biol.* 7, 935-941.
- (9) Thomson, A. R., Wood, C. W., Burton, A. J., Bartlett, G. J., Sessions, R. B., Brady, R. L., and Woolfson, D. N. (2014) Computational design of water-soluble alpha-helical barrels. *Science* 346, 485-488.
- (10) Aronsson, C., Danmark, S., Zhou, F., Oberg, P., Enander, K., Su, H. B., and Aili, D. (2015) Self-sorting heterodimeric coiled coil peptides with defined and tuneable self-assembly properties. *Sci. Rep.* 5.
- (11) Jin, J., Baker, E. G., Wood, C. W., Bath, J., Woolfson, D. N., and Turberfield, A. J. (2019) Peptide Assembly Directed and Quantified Using Megadalton DNA Nanostructures. *ACS Nano* 13, 9927-9935.
- (12) Thomas, F., Boyle, A. L., Burton, A. J., and Woolfson, D. N. (2013) A Set of de Novo Designed Parallel Heterodimeric Coiled Coils with Quantified Dissociation Constants in the Micromolar to Sub-nanomolar Regime. *J. Am. Chem. Soc.* 135, 5161-5166.
- (13) Fairman, R., Chao, H. G., Mueller, L., Lavoie, T. B., Shen, L. Y., Novotny, J., and Matsueda, G. R. (1995) Characterization of a New 4-Chain Coiled-Coil - Influence of Chain-Length on Stability. *Protein Sci.* 4, 1457-1469.
- (14) Burton, A. J., Thomson, A. R., Dawson, W. M., Brady, R. L., and Woolfson, D. N. (2016) Installing hydrolytic activity into a completely de novo protein framework. *Nat. Chem.* 8, 837-844.
- (15) Fletcher, J. M., Horner, K. A., Bartlett, G. J., Rhys, G. G., Wilson, A. J., and Woolfson, D. N. (2018) De novo coiled-coil peptides as scaffolds for disrupting protein-protein interactions. *Chem. Sci.* 9, 7656-7665.
- (16) Rhys, G. G., Wood, C. W., Beesley, J. L., Zaccai, N. R., Burton, A. J., Brady, R. L., Thomson, A. R., and Woolfson, D. N. (2019) Navigating the Structural Landscape of De Novo alpha-Helical Bundles. *J. Am. Chem. Soc.* 141, 8787-8797.
- (17) Gonzalez, L., Woolfson, D. N., and Alber, T. (1996) Buried polar residues and structural specificity in the GCN4 leucine zipper. *Nat. Struct. Biol.* 3, 1011-1018.
- (18) Swainsbury, D. J. K., Harniman, R. L., Di Bartolo, N. D., Liu, J. T., Harper, W. F. M., Corrie, A. S., and Jones, M. R. (2016) Directed assembly of defined oligomeric photosynthetic reaction centres through adaptation with programmable extra-membrane coiled-coil interfaces. *Biochim. Biophys. Acta, Bioenerg.* 1857, 1829-1839.
- (19) Sciore, A., Su, M., Koldewey, P., Eschweiler, J. D., Diffley, K. A., Linhares, B. M., Ruotolo, B. T., Bardwell, J. C. A., Skiniotis, G., and Marsh, E. N. G. (2016) Flexible, symmetry-directed approach to assembling protein cages. *Proc. Natl. Acad. Sci. U.S.A.* 113, 8681-8686.
- (20) Tavenor, N. A., Murnin, M. J., and Horne, W. S. (2017) Supramolecular Metal-Coordination Polymers, Nets, and Frameworks from Synthetic Coiled-Coil Peptides. *J. Am. Chem. Soc.* 139, 2212-2215.
- (21) Burgess, N. C., Sharp, T. H., Thomas, F., Wood, C. W., Thomson, A. R., Zaccai, N. R., Brady, R. L., Serpell, L. C., and Woolfson, D. N. (2015) Modular Design of Self-Assembling Peptide-Based Nanotubes. *J. Am. Chem. Soc.* 137, 10554-10562.
- (22) Lombardi, A., Pirro, F., Maglio, O., Chino, M., and DeGrado, W. F. (2019) De Novo Design of Four-Helix Bundle Metalloproteins: One Scaffold, Diverse Reactivities. *Accounts Chem. Res.* 52, 1148-1159.
- (23) Liu, J., Zheng, Q., Deng, Y. Q., Cheng, C. S., Kallenbach, N. R., and Lu, M. (2006) A seven-helix coiled coil. *Proc. Natl. Acad. Sci. U.S.A.* 103, 15457-15462.
- (24) Deng, Y. Q., Liu, J., Zheng, Q., Eliezer, D., Kallenbach, N. R., and Lu, M. (2006) Antiparallel four-stranded coiled coil specified by a 3-3-1 hydrophobic heptad repeat. *Structure* 14, 247-255.
- (25) Smith, A. J., Thomas, F., Shoemark, D., Woolfson, D. N., and Savery, N. J. (2019) Guiding Biomolecular Interactions in Cells Using de Novo Protein-Protein Interfaces. *ACS Synth. Biol.* 8, 1284-1293.
- (26) Walshaw, J., and Woolfson, D. N. (2003) Extended knobs-into-holes packing in classical and complex coiled-coil assemblies. *J. Struct. Biol.* 144, 349-361.
- (27) Crick, F. H. C. (1953) The Packing of Alpha-Helices - Simple Coiled-Coils. *Acta Crystallogr.* 6, 689-697.

TOC Figure:

